A THESIS FOR THE DEGREE OF MASTER

The assessment of MMP-9 and clinical characteristics in dogs with tracheobronchomalacia based on cough severity and fluoroscopy

기관기관지연화증에 이환된 개에서 기침의 중증도와 투시검사에 기초한 임상적 특징과 MMP-9의 평가

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이 논문을 수의학 석사 학위논문으로 제출함

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Abstract

MMP-9 and clinical characteristics in dogs with tracheobronchomalacia based on cough severity and fluoroscopy

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Tracheobronchomalacia (TBM), a common disease in dogs, is characterized by cough; however, little is known about the serum biomarkers that can objectively evaluate the severity of cough in canine TBM. The objective of this study was to evaluate the relationship between cough severity and clinical characteristics, fluoroscopic images, and new serum biomarkers in canine TBM.

Fifty-one client-owned dogs diagnosed with TBM based on fluoroscopy and clinical signs were enrolled in this cross-sectional study. These dogs were divided into three groups according to the severity of cough (grade of cough: 0, 1, and 2). Signalments, comorbidities, and fluoroscopic characteristics were compared among the groups. The serum matrix metalloproteinase-9 (MMP-9), interleukin-6

(IL-6), surfactant protein-A (SP-A), and syndecan-1 (SDC-1) levels were measured in all groups.

No significant differences in age, breed, sex, or clinical history were observed among the groups. Concomitant pharyngeal collapse increased significantly with the severity of cough ($p < 0.05$). Based on the fluoroscopic characteristics, the TBM grade of the carinal region increased significantly and consistently with the grade of cough ($p < 0.05$). The serum MMP-9 level was significantly higher in the grade 2 group than that in the grade 0 group ($p < 0.05$). The serum IL-6 level was significantly lower in the grade 1 group than that in the grade 0 group ($p < 0.05$). The serum SP-A and SDC-1 levels did not differ significantly among the groups.

With further studies, MMP-9 may be used as an objective serum biomarker that represents cough severity to understand the pathogenesis.

Keywords: Dog, Fluoroscopy, IL-6, MMP-9, Tracheobronchomalacia, Tracheal collapse,

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1. Introduction

Tracheal collapse (TC) is a common disease in small-breed dogs that causes chronic cough due to the flattening of the tracheal cartilage and tracheal membrane prolapse into the lumen [1, 2]. Similar to human medicine, the term tracheobronchomalacia (TBM) has been used recently in veterinary medicine to describe the involvement of the bronchi along with trachea in TC [2]. TBM can occur due to congenital or secondary causes, and chronic inflammation or other factors can exacerbate the clinical signs [1-5]. However, the pathophysiology of TBM and the inflammatory mediators involved in the disease progression are not completely understood [2, 6]. TBM is diagnosed via radiography, fluoroscopy, or tracheobronchoscopy on the basis of the clinical signs [6]. The grading of TBM is based on the percentage reduction in the luminal diameter [6, 7]. TBM has been observed in several dogs with no history of cough [4], and the clinical features were not predictive of airway collapse [8]. Reportedly, the symptom-free period of TBM has no correlation with sex, age, or the findings on the fluoroscopic images [9]; however, little is known about the relevance of severity and TBM grade.

Although the definitive cause of TBM in human beings is unknown, nearly half of the human patients with TBM have chronic obstructive pulmonary disease (COPD) [6, 10, 11]. COPD is a chronic inflammatory lung disease that causes irreversible airway obstruction [12, 13]. Canine TBM and human COPD differ from each other due to anatomical differences; however, the inflammation and structural changes resulting in airflow limitation in human COPD are similar to those of canine

TBM. The Global Initiative for Chronic Obstructive Lung Disease criteria grades, which are determined by the percentage of forced expiratory volume in one second, are used to evaluate COPD [14, 15]. However, there are no established indicators to evaluate the clinical symptoms in dogs with TBM. Several serum biomarkers and inflammatory factors of COPD are being investigated due to the complexity of COPD [16, 17]. In contrast, studies investigating the serum biomarkers of TBM in both humans and dogs are lacking. Several biomarkers secreted by various cells in the lungs, including matrix metalloproteinase-9 (MMP-9), interleukin-6 (IL-6), surfactant protein-A (SP-A), and Syndecan-1 (SDC-1), have been identified for COPD in humans. The serum MMP-9, IL-6, and SP-A levels are increased in patients with COPD, whereas that of SDC-1 is decreased [13, 18-22]. However, few studies have investigated the role of these biomarkers in canine TBM.

This study evaluated the serum concentrations of MMP-9, IL-6, SP-A, and SDC-1 and the fluoroscopic characteristics of dogs with TBM to determine their correlation with the severity of cough. This study aimed to 1) identify a serum indicator that objectively represents the severity of TBM in dogs, and 2) determine whether the fluoroscopic characteristics at the time of diagnosis were related to the severity of cough.

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2. Materials and Methods

2.1. Case selection

Among the cases that presented to the Veterinary Medical Teaching Hospital (VMTH) between August 2022 and December 2022, 51 dogs previously diagnosed with TBM based on the findings on the fluoroscopic images and the clinical signs were enrolled in this cross-sectional study. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (SNU) (approval number: SNU-221208)

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows:1) any age and breed of dogs; 2) cases diagnosed with TBM based on the findings on the fluoroscopic images and the clinical signs observed previously at VMTH of SNU; and 3) cases with cough regardless of the severity. Notably, cases with no cough at the time of the clinical visit were also included.

The exclusion criteria were 1) cases with comorbidities that affected the lung parenchyma, such as asthma, pneumonia, and pulmonary mass; 2) cases suspected to have TBM but not diagnosed by fluoroscopy; and 3) cases in which the cough severity was unavailable on the day of the clinical visit.

2.3. Data collection for clinical evaluation

The medical records of the dogs enrolled in this study were acquired on the day of their clinical visit. The following information was collected: signalment; severity of cough; fluoroscopy; comorbidity, including Myxomatous Mitral Valve Disease (MMVD), American College of Veterinary Internal Medicine (ACVIM) stage; and medication history.

2.4. Diagnosis of TBM

TBM was diagnosed based on the findings on the fluoroscopic images and the clinical signs at the time of diagnosis. Fluoroscopy of the normal respiration phase and the forced expiration phase (cough phase) was performed. The grade of TBM by location (cervical, thoracic, intrathoracic, and carina) and the presence of tracheal kinking or bronchial collapse were evaluated by radiologists. TBM was graded based on the percentage reduction in the luminal diameter as follows: grade 1, 0–25%; grade 2, 25–50%; grade 3, 50–75%; and grade 4, > 75% [4, 6, 9, 23, 24].

2.5. Evaluation of the severity of cough

Since there are no objective methods or subjective questionnaires to evaluate the frequency or severity of cough in veterinary medicine, following standard were created to grade the severity of cough: grade 0, no cough; grade 1, frequency less than three times a day; grade 2, frequency more than three times a day, duration of

more than 5 minutes at a time, or a severe cough accompanied by cyanosis. Information regarding the frequency and severity of the cough was obtained from the owners on the day of the clinical visit, and the dogs were divided into three groups according to these grades.

2.6. Sample collection and preparation

Blood samples were collected from the jugular or cephalic vein by a veterinarian and stored in tubes without anticoagulants on the day of the clinical visit. After the required hematological examination was performed to manage the concomitant diseases, the remaining blood was collected. The serum was extracted by centrifugation at 4000 × g for 3 minutes at 4°C and stored in Eppendorf tubes at -80°C. These serum samples were defrosted at 37°C as required for enzyme-linked immunosorbent assay (ELISA) analysis.

2.7. Measurement of the serum MMP-9, IL-6, SP-A, and SDC-1 levels

The serum MMP-9, IL-6, SP-A, and SDC-1 levels were measured using ELISA according to the manufacturer's protocol. Canine MMP-9, SP-A, and SDC-1 ELISA kits were purchased from MyBioSource Inc. (San Diego, CA, USA). Canine IL-6 ELISA kits were purchased from RnDSystems Inc. (Minneapolis, MN). Standard dilutions for all kits were performed according to the manufacturer's instructions, and all samples were run in duplicate. The optical density (OD) of all samples was read at 450 nm using a microplate reader, and the mean value of the

duplicates was calculated.

2.8. Statistical analysis

Statistical analyses of the data were performed using GraphPad Prism (version 9.5.0, GraphPad Inc., San Diego, CA) and SPSS (version 29.0, IBM SPSS Inc., Chicago, IL). Normality tests were performed using the Shapiro–Wilk test. Based on the results of the normality test, nonparametric tests, including the Mann–Whitney U test, Kruskal–Wallis test, and Chi-square test, were used to evaluate the differences among the groups. Fisher's exact test was used instead of the chi-square test when more than 20% of the expected cell counts were less than 5. In all comparisons, p-values < 0.05 were considered statistically significant. All descriptive statistics for continuous variables are presented as medians (range).

3. Results

3.1. Patient data

Fifty-one dogs with TBM that met the inclusion criteria were enrolled in this study and classified into three groups according to the severity of cough. Fifteen, 18, and 18 dogs were grouped in grades 0, 1, and 2, respectively. The clinical characteristics of the dogs in each group are presented in Table 1. The following breeds of dogs with TBM were included: Pomeranian (n = 15, 29.4%), Maltese (n = 13, 25.5%), Toy and Miniature Poodle (n = 8, 15.7%), mixed (n = 4, 7.8%), Chihuahua (n = 4, 7.8%), and Shihtzu (n = 3, 5.9%). One dog (2.0%) of each of the following breeds was also included: Beagle, Silky Terrier, Spitz, and Yorkshire terrier. The median age of the dogs was 12 years (range, 4–17 years). Twenty-two female (43.1%, three intact females, 19 spayed females) and 29 male (56.8%, one intact male, 28 castrated males) dogs were included. No significant differences in the breed, age or sex were observed among the groups.

Dogs with comorbidities accounted for 96.1% (n = 49), and only 3.9% (n = 2) of the dogs were managed for TBM alone. Comorbid diseases that can induce cough included MMVD (n = 36, 70.6%), soft palate elongation or thickening (n = 20, 39.2%), and pharyngeal collapse (n = 21, 41.2%). The ACVIM stages of the 36 dogs with MMVD were B1 (n = 7, 13.7%), B2 (n = 13, 25.5%), and C (n = 16, 431.4%). The number and percentage of dogs with each comorbidity in each group are summarized in Table 1. The percentage of dogs with concomitant pharyngeal

collapse increased significantly with cough severity ($p < 0.05$). No significant difference was observed between the concomitance of MMVD, MMVD ACVIM stage, and soft palate elongation or thickening among the cough groups. All dogs with comorbidities were treated or managed at the time of blood sampling.

At the time of evaluation, 39.2% (n = 20) of the dogs were not receiving treatment for TBM, whereas 60.8% (n = 31) of the dogs were receiving medication for TBM. The medications used included theophylline (n = 31, 60.8%), codeine (n = 14, 27.5%), montelukast (n = 3, 5.9%), tulobuterol patch (n = 3, 5.9%), bromhexine (n = 2, 3.9%), salbutamol (n = 2, 3.9%), fluticasone inhaler (n = 2, 3.9%), salbutamol nebulization (n = 1, 2.0%), and prednisolone (n = 1, 2.0%). All dogs under treatment for TBM were receiving theophylline as the first-line drug and codeine as the second-line drug, and 13.7% (n = 7) of the dogs were receiving more than three medications. The number and percentage of dogs receiving each medication in the three cough groups are summarized in Table 1. The usage of drug, including those of theophylline ($p < 0.05$) and codeine ($p < 0.001$), increased significantly as the grade of cough increased. No significant differences in the percentage of patients receiving three or more drugs were observed among the three grades of cough.

The clinical history was defined as the period from the day of diagnosis to the day of clinical evaluation and blood collection. Based on the clinical history, the participants were categorized into three groups: < 3 months (n = 18, 35.3%), 3–6 months (n = 2, 3.9%), and > 6 months (n = 31, 60.8%). The number and percentage of dogs corresponding to each clinical history group in all cough-grade groups are summarized in Table 1. No significant relationship between the clinical history and the severity of cough was observed among the three cough-grade groups.

3.2. Fluoroscopic characteristics in all cough groups

Fluoroscopic images were obtained on the day of diagnosis for all 51 dogs. The fluoroscopic grades of the cervical, thoracic, intrathoracic, and carinal regions were evaluated in all cough groups. TBM was detected in the thoracic (84.3%, n = 43), intrathoracic (90.2%, n = 46), and carina (96.1%, n = 49) regions in most cases, whereas TBM was detected in the cervical region (41.2%, n = 21) in less than half of the cases. The fluoroscopic TBM grades of the cervical region (Fig. 1A), thoracic region (Fig. 1B), and intrathoracic region (Fig. 1C) were not associated with the grade of cough. In contrast, the fluoroscopic TBM grade of the carinal region increased significantly with the severity of cough ($p < 0.05$, Fig. 1D). Bronchial collapse was observed in 49% (n = 25) of the dogs, lung herniation in 70.6% (n = 36), and tracheal kinking in 25.5% (n = 13). The presence or absence of bronchial collapse (Fig. 1E), tracheal kinking (Fig. 1F), and lung herniation (Fig. 1G) did not differ significantly among the cough-grade groups.

3.3. Serum levels of MMP-9, IL-6, SP-A, and SDC-1 in all cough groups

Serum levels of MMP-9, IL-6, SP-A, and SDC-1 in all cough groups were evaluated. Median and range of each serum factor concentration in all groups, and the *p*-value between the groups are summarized in Table 2.

The serum MMP-9 (median, range, ng/mL) level was significantly higher in the grade 2 group (1.54, 0.80–4.69 ng/mL) than that in the grade 0 group (0.91,

0.53–1.69 ng/mL) ($p < 0.05$, Fig. 2A). No significant difference was observed between the grade 1 group (1.20, 0.62–11.78 ng/mL) and other groups.

The serum IL-6 (median, range, pg/mL) level was significantly lower in the grade 1 group (53.10, 46.49–62.92 pg/mL) than that in the grade 0 group (68.93, 48.09—220.42 pg/mL) [$p < 0.05$, Fig. 2B]. There were no significant differences between the grade 2 group (51.70, 46.09–78.55 pg/mL) and other groups.

The serum SP-A (median, range, ng/mL) level did not differ significantly among the groups: grade 0 (2.45, 2.12–4.81 ng/mL), grade 1 (2.68, 2.31–4.97 ng/mL), and grade 2 (2.51, 2.12–4.81 ng/mL) (Fig. 2C).

The serum SDC-1 (median, range, ng/mL) level did not differ significantly among the groups: grade 0 (2.40, 0.57–2.79 ng/mL), grade 1 (2.37, 0.57–2.71 ng/mL), and grade 2 (2.28, 0.57–5.14 ng/mL) (Fig. 2D).

Since the concomitance of pharyngeal collapse increased significantly with the clinical severity of cough, the relationship between pharyngeal collapse and the levels of MMP-9 and IL-6 were evaluated. No significant difference in the serum levels of MMP-9 was observed among the cough-grade groups, regardless of the presence of concomitant pharyngeal collapse: in grade 0 (Fig. 3A), in grade 1 (Fig. 3B), and in grade 2 (Fig. 3C). Similarly, no significant differences in the serum levels of IL-6 were observed within the cough-grade groups, regardless of the presence of concomitant pharyngeal collapse: in grade 0 (Fig. 3D), in grade 1 (Fig. 3E), and in grade 2 (Fig. 3F).

4. Discussion

In the present study, the signalments, comorbidities, fluoroscopic characteristics, and serum biomarkers (MMP-9, IL-6, SP-A, and SDC-1) of 51 dogs with TBM with different cough grades were investigated. Age, breed, sex, and clinical history were not related to the severity of cough. Patients with concurrent pharyngeal collapse had significantly higher grades of cough than that of those without pharyngeal collapse. Among the various fluoroscopic characteristics, only the TBM grade of the carinal region was related to the severity of cough. The serum MMP-9 level was positively correlated with the grade of cough, whereas the serum IL-6 level was negatively correlated with the grade of cough.

The clinical signs of canine TBM are mostly described as a harsh, dry, and honking cough, which wax and wane or occurs paroxysmally. Moreover, the cough is often initiated by an acute-on-chronic event [2, 6]. The Cough Symptom Score for humans consists of a two-part questionnaire (daytime and night-time symptoms). The responses range from 0–5; thus, the total score ranges from 0 (no cough) to 10 (most severe cough) [25, 26]. However, there is no consensus regarding a questionnaire for cough in dogs; therefore, the dogs enrolled in this study were divided into the following groups based on the frequency of cough: more than three times a day, more than 5 minutes of cough, or a severe cough accompanied by cyanosis noticed by owners that lowers the quality of life. In human patients with COPD, the objective and subjective evaluation of symptoms is possible through pulmonary function tests and questionnaires; thus, the patient's condition can be

evaluated relatively accurately. However, forced expiratory volume, which plays a crucial role in pulmonary function tests, cannot be measured voluntarily in dogs, and there are no established methods for the objective evaluation of the clinical signs of TBM [27, 28]. Currently, the management of TBM in dogs focuses on history taking; therefore, objective indicators for evaluating the disease status are required. Through this study, I aimed to advance the management of TBM in dogs and provide a basis for understanding the etiology of TBM in the future.

Similar to previous studies, most of the 51 dogs enrolled in this study were middle-aged or older small-breed dogs, and there was no sex predilection [6, 23]. Overrepresented breeds included Pomeranians, Maltese, Toy and Miniature Poodles, mixed-breed dogs, and Chihuahuas. Age, breed, and sex were found to have no relationship with the grades of cough in the present study. Moreover, no significant differences were observed in the clinical history among the cough-grade groups. The use of theophylline and codeine increased significantly with the severity of cough. All dogs under treatment for TBM were receiving theophylline as the first-line drug and codeine as the second-line drug in this study. Most of the dogs with TBM in the current study were middle-aged or older and had comorbidities, such as endocrine diseases, liver enzyme elevation, and chronic pancreatitis, which made it difficult to administer corticosteroid, the conventional first-line anti-inflammatory drug, repeatedly or for a long period [9]. The increased use of theophylline and codeine with the increase in the grade of cough may be due to the tendency of veterinarians to prescribe these medications with the aggravation of clinical signs.

Our data demonstrated that concomitant pharyngeal collapse increased significantly with the severity of cough. In a previous study, 60.7% of dogs with

pharyngeal collapse had TBM as a comorbidity [29]. In the present study, pharyngeal collapse was concomitant in 41.2% of dogs with TBM, suggesting that TBM and pharyngeal collapse are associated. In dogs with TBM, the pressure gradient between the upper and lower airways is increased during respiration due to the narrowing of the airway, which increases resistance within the lumen [29, 30]. This altered pressure gradient imposes a chronic load and changes the tone of the dilator muscles of the pharynx, which may hinder the maintenance of the normal pharyngeal anatomy, thereby leading to pharyngeal collapse [29, 31, 32]. Cough increases the respiratory pressure gradient further [33], which may accelerate this further. Pharyngeal collapse induces pharyngeal contraction, further worsening the cough, creating a vicious circle [34]. Dogs previously diagnosed with concurrent pharyngeal collapse had a more severe cough in this study; thus, concurrent pharyngeal collapse can be a risk factor for the progression of TBM.

The presence of concomitant MMVD had no relationship with the severity of cough. Cough is one of the main clinical signs in dogs with MMVD, which may be a result of the enlarged left atrium stimulating the cough receptors by imposing mechanical pressure on the airways [35]. A previous study reported that airway collapse was already present in all MMVD dogs with cough regardless of the enlargement of the left atrium, and there was no significant relationship between left atrial enlargement and the distribution of airway collapse [36]. Thus, cough in dogs with MMVD may not be related to airway collapse. Similarly, in the present study, concomitance or stages of MMVD had no significant relationship with the grade of cough in dogs with TBM. This further shows that the concomitance of MMVD does not affect the severity of cough in canine TBM. The concomitance of soft palate

elongation and thickening had no relationship with the severity of cough. A previous study reported that concurrent soft palate elongation was found in 7.6% of dogs with TBM. In contrast, in the present study, soft palate elongation or thickening was found in 39.2% of dogs with TBM [37].

I also assessed the relationship between fluoroscopic characteristics and the grade of cough. Among the cervical, thoracic, intrathoracic, and carinal regions, only the TBM grade of the carinal region increased significantly with the severity of cough. The collapse of the cervical and thoracic regions occurs during inspiration. Similarly, the collapse of the intrathoracic and carinal regions occurs during expiration. These occur due to pressure differences within the airway during respiration [6, 23]. In previous studies, the percentage of dogs with TBM presenting with collapse in each tracheal region was as follows: cervical, 16–55.3%; thoracic, 60.5–80.9%; intrathoracic, 86.9–91.5%; and carinal, 93.5– 95.7% [9, 23]. Similarly, in the present study, the percentage of collapse in the tracheal region increased sequentially from the cranial to caudal regions as follows: cervical, 41.2%; thoracic, 84.3%; intrathoracic, 90.2%; and carinal, 96.1%. A previous study reported that a history of cough was not related to TBM, and many cases had fluoroscopic TBM without a history of cough [4]. However, the severity of cough increased significantly with the TBM grade of the carinal region in the present study. This may be related to the differences in the thicknesses of the cartilage and muscle, as the thickness of the ventral midpoint cartilage and the tracheal muscle decreases gradually from the cervical to the intrathoracic region [38]. Further studies are required to determine whether any special characteristics of the carinal region enable an easier collapse and why only the carinal region is related to the severity of cough.

Bronchial collapse, tracheal kinking, and lung herniation were found to have no relationship with the grade of cough. It is recognized that abnormalities of the cartilage of the bronchus cause bronchial collapse in dogs with TBM [6, 8]. Similar to previous studies that reported bronchial collapse in 45.8– 83% of the dogs with TBM, 49% of the dogs with TBM had fluoroscopic bronchial collapse in the present study [6, 8, 37]. To the best of the authors' knowledge, little is known about the relationship between bronchial collapse and cough [39]. The results of this study showed that no significant differences were observed among the groups in terms of the presence of bronchial collapse, suggesting that bronchial collapse does not worsen the cough. Another study has reported that airway inflammation is not related to bronchial collapse [8], and more studies are required to identify cases in which bronchial collapse accompanies TBM in dogs. Increased thoracic muscle weakness with aging and increased intrathoracic pressure may cause cervical lung herniation [4]. Previous studies have reported cervical lung herniation in 55.9–70% of all dogs that underwent fluoroscopy. Positive relationship between lung herniation and age, the presence of intrathoracic TC, bronchial collapse, and tracheal kinking has also been revealed [4, 40]. In the present study, lung herniation was observed in 70.6% of dogs with TBM, and it had no relationship with the severity of cough. This is consistent with the findings of a previous study that found no association between lung herniation and chronic cough [4], suggesting that lung herniation may be the result of TBM. The presence of lung herniation does not further exacerbate the clinical presentation; however, further studies are required to reveal the exact relationship between lung herniation and TBM. Lastly, previous studies have reported that tracheal kinking was found in 27–29.3% of all dogs that underwent

fluoroscopy [4, 40]. In the present study, tracheal kinking was observed in 25.5% of dogs with TBM and had no relationship with the grade of cough. This was in contrast with our hypothesis that tracheal kinking would aggravate the symptoms by causing damage to the trachea. In human patients with acquired TBM, kinking occurs at the transition between the malacic tracheal wall and the normal segment [41]; however, the exact cause of tracheal kinking in dogs with TBM has not been revealed, but the weakened cartilage and increased airway resistance due to TBM might be the cause. Although tracheal kinking does not manifest as cough, further studies on the factors that predispose dogs with TBM to tracheal kinking are required.

A limited number of studies have investigated serum biomarkers of TBM in dogs; therefore, novel serum biomarkers of COPD that are being actively studied were selected [13, 18-22]. MMP-9, IL-6, SP-A, and SDC-1 were selected as the serum biomarkers, and the serum level of each factor in all cough-grade groups was evaluated. While the levels of SP-A and SDC-1 showed no significant differences among the cough-grade groups, the MMP-9 level was significantly higher in the grade 2 group compared with that in the grade 0 group. In contrast, IL-6 was significantly decreased in the grade 1 group compared with that in the grade 0 group. No differences in the MMP-9 and IL-6 levels were observed within each cough-grade group, regardless of concomitant pharyngeal collapse.

MMP-9, also known as 92 kDa type IV collagenase, is the predominant protease in the alveolar tissue. Due to its easy detection and quantification, it has attracted attention among MMPs [13, 20]. There is increasing evidence suggesting that MMPs are involved in the pathogenesis of COPD [42], and recent studies have shown that MMPs and their inhibitors play a central role in lung remodeling in

COPD [20, 43]. The levels of several pro-inflammatory cytokines and MMP-9 are increased during the acute inflammatory response of COPD. In addition, MMP-9 is secreted by the alveolar type II cells, alveolar macrophages, neutrophils, bronchial epithelial cells, Clara cells, endothelial cells, fibroblasts, and smooth muscle cells in the lung [13, 20]. MMP-9 degrades elastin and promotes further lung damage and is suggested as a key mediator in COPD [13]. The serum level of MMP-9 increases with the clinical severity and the duration of clinical history in patients with COPD [13]. This finding is similar to the result of the present study that showed a positive correlation between the serum MMP-9 level and the severity of cough in dogs with TBM. Further studies are required to determine whether the serum MMP-9 level is increased as a result of inflammation induced by cough or whether the increase in the pathway of inflammation induces cough.

IL-6 is a key cytokine in inflammatory storms that acts as a pro-inflammatory mediator and acute phase response inducer [19, 44]. IL-6 can be produced by different sources in the lung, such as epithelial cells, interstitial fibroblasts, macrophages, and other inflammatory cells [45]. IL-6 is produced downstream from the response to a variety of stimuli, such as allergens, respiratory viruses, exercise, environmental particles, and inhaled toxic particles [45]. IL-6 contributes to lung damage through mucus hypersecretion, matrix deposition, and protease release from granulocytes via regulatory mechanisms [44, 45]. Studies have revealed that the serum IL-6 level is elevated in patients with COPD, especially during the acute exacerbation phase [18, 44]. IL-6 antibody has been proposed as a novel therapeutic agent for improving airflow limitation due to IL-6-induced airway mucus hypersecretion in patients with COPD [44]. In veterinary medicine, novel

supplement that can alleviate inflammation and oxidative stress, improved clinical signs and decreased the IL-6 and tumor necrosis factor-α (TNF-α) levels, suggesting that TC induces the synthesis and secretion of pro-inflammatory cytokines [5]. Therefore, I hypothesized that IL-6 would increase with the severity of cough; however, IL-6 was found to be negatively correlated with the severity of cough in dogs with TBM, possibly due to the anti-inflammatory properties of IL-6 which inhibit TNF-α and IL-1 and decrease the IL-6 levels in decreased anti-inflammatory mechanisms [19]. Further studies are required to determine whether other factors decrease IL-6 in dogs with TBM with cough compared with dogs without cough. Moreover, similar to the findings of a previous study, the serum IL-6 level was decreased in patients with idiopathic COPD compared with that of controls. Further studies that compare the IL-6 level in healthy dogs and dogs with TBM would help reveal the role of IL-6 in dogs with TBM [46].

SP-A is a pulmonary surfactant that enhances pathogen clearance and regulates adaptive and innate immune-cell functions [47, 48]. SP-A functions as an opsonin by binding to a variety of bacteria, viruses, allergens, and apoptotic cells, and is secreted by alveolar type II cells and Clara cells [47-49]. SP-A also has direct effects on immune cells modulating the production of cytokines and inflammatory mediators [47]. At present, SP-A has been established to have a relationship with COPD in both animal and human studies and may be related to the progression and prognostic evaluation of COPD in terms of airway remodeling, inflammatory response, and clinical symptoms [21]. In a previous study, the serum SP-A levels were negatively correlated with pulmonary function tests and positively correlated with inflammatory indicators and clinical severity in patients with COPD [21].

However, the present study revealed that SP-A was not related to the severity of cough in dogs with TBM.

SDC-1 is the main proteoglycan of the airway epithelial cells and plays an important role in the inflammatory process [22, 50]. SDC-1 controls epithelial plasticity and promotes fibroproliferation by altering the alveolar epithelium to a profibrotic phenotype [51]. SDC-1 expression facilitates cytoprotective signals and helps limit inflammation, thereby minimizing lung injury [52]. The serum SDC-1 level is decreased in patients with COPD and has a negative correlation with lung function, exacerbation risk, and systemic inflammation [22]. However, the present study revealed that SDC-1 was not related to the severity of cough in dogs with TBM.

This study had several limitations. First, the number of dogs enrolled in this study was limited, and no control group was included. A larger population with matched healthy controls is required to confirm the findings of the present study. Second, the fluoroscopic characteristics do not represent the case at the time of the clinical visit as the fluoroscopic images were acquired at the time of diagnosis and not followed up. Lastly, most cases in this study had comorbidities, which may have affected serum factors, as other diseases can also induce inflammation. Therefore, further controlled studies on MMP-9 and IL-6 are required in the future.

5. Conclusion

The concomitance of pharyngeal collapse may be a risk factor for the progression of canine TBM. Based on various fluoroscopic characteristics, the TBM grade of the carinal region can be the major TBM grade that predicts the severity of cough. With further studies, MMP-9 may be used as a new serum biomarker to objectively represent the severity of cough in canine TBM. Additionally, both MMP-9 and IL-6 may be used to understand the progression and management of TBM.

6. References

1. Cote E, Cohn L: Cote's Clinical veterinary Advisor: Dogs & Cats, 4th edn: Mosby; 2019.

2. Nelson RW, Couto CG: Small Animal Internal Medicine, 6th edn: Elsevier - Health Sciences Division; 2019.

3. Hawkins EC, Clay LD, Bradley JM, Davidian M: Demographic and Historical Findings, Including Exposure to Environmental Tobacco Smoke, in Dogs with Chronic Cough. *J Vet Intern Med* 2010, 24(4):825-831.

4. Lee J, Yun S, Lee I, Choi M, Yoon J: Fluoroscopic characteristics of tracheal collapse and cervical lung herniation in dogs: 222 cases (2012-2015). *J Vet Sci* 2017, 18(4):499-505.

5. Mektrirat R, Rueangsri T, Keeratichandacha W, Soonsawat S, Boonyapakorn C, Pongkan W: Polyunsaturated Fatty Acid EAB-277((R)) Supplementation Improved Heart Rate Variability and Clinical Signs in Tracheal Collapse Dogs. *Front Vet Sci* 2022, 9:880952.

6. Della Maggiore A: An Update on Tracheal and Airway Collapse in Dogs. *Veterinary Clinics of North America: Small Animal Practice* 2020, 50(2):419-430.

7. Tangner CH, Hobson HP: A Retrospective Study of 20 Surgically Managed Cases of Collapsed Trachea. *Veterinary surgery* 1982, 11(4):146-149.

8. Johnson LR, Pollard RE: Tracheal collapse and bronchomalacia in dogs: 58 cases (7 /2001-1 /2008). *J Vet Intern Med* 2010, 24(2):298-305.

9. Jeung SY, Sohn SJ, An JH, Chae HK, Li Q, Choi M, Yoon J, Song W-J, Youn HY: A retrospective study of theophylline-based therapy with tracheal collapse in small-breed dogs: 47 cases (2013-2017). *J Vet Sci* 2019, 20(5):e57-e57.

10. Majid A, Sosa AF, Ernst A, Feller-Kopman D, Folch E, Singh AK, Gangadharan S: Pulmonary function and flow-volume loop patterns in patients with tracheobronchomalacia. *Respir Care* 2013, 58(9):1521-1526.

11. Biswas A, Jantz MA, Sriram PS, Mehta HJ: Tracheobronchomalacia. *Disease-a-Month* 2017, 63(10):287-302.

12. Chen Y, Chen P, Hanaoka M, Droma Y, Kubo K: Enhanced levels of prostaglandin E2 and matrix metalloproteinase-2 correlate with the severity of airflow limitation in stable COPD. *Respirology* 2008, 13(7):1014-1021.

13. Verma AK, Pandey AK, Singh A, Kant S, Mahdi AA, Prakash V, Ansari KM, Dixit RK, Chaudhary SC: Increased Serum Levels of Matrix-metalloproteinase-9, Cyclo-oxygenase-2 and Prostaglandin E-2 in Patients

with Chronic Obstructive Pulmonary Disease (COPD). *Indian J Clin Biochem* 2022, 37(2):169-177.

14. Gil HI, Zo S, Jones PW, Kim BG, Kang N, Choi Y, Cho HK, Kang D, Cho J, Park HY *et al*: Clinical Characteristics of COPD Patients According to COPD Assessment Test (CAT) Score Level: Cross-Sectional Study. *Int J Chron Obstruct Pulmon Dis* 2021, 16:1509-1517.

15. Venkatesan P: GOLD COPD report: 2023 update. *Lancet Respir Med* 2023, 11(1):18.

16. Pantazopoulos I, Magounaki K, Kotsiou O, Rouka E, Perlikos F, Kakavas S, Gourgoulianis K: Incorporating Biomarkers in COPD Management: The Research Keeps Going. *Journal of Personalized Medicine* 2022, 12(3):379.

17. Hollander Z, DeMarco ML, Sadatsafavi M, McManus BM, Ng RT, Sin DD: Biomarker Development in COPD: Moving From P Values to Products to Impact Patient Care. *Chest* 2017, 151(2):455-467.

18. Yang D, Wang L, Jiang P, Kang R, Xie Y: Correlation between hs-CRP, IL-6, IL-10, ET-1, and Chronic Obstructive Pulmonary Disease Combined with Pulmonary Hypertension. *J Healthc Eng* 2022, 2022:3247807.

19. Hussein FGM, Mohammed RS, Khattab RA, Al-Sharawy LA: Serum interleukin-6 in chronic obstructive pulmonary disease patients and its relation to severity and acute exacerbation. *The Egyptian Journal of Bronchology* 2022, 16(1).

20. Li Y, Lu Y, Zhao Z, Wang J, Li J, Wang W, Li S, Song L: Relationships of MMP-9 and TIMP-1 proteins with chronic obstructive pulmonary disease risk: A systematic review and meta-analysis. *J Res Med Sci* 2016, 21:12.

21. Lv MY, Qiang LX, Wang BC, Zhang YP, Li ZH, Li XS, Jin LL, Jin SD: Complex Evaluation of Surfactant Protein A and D as Biomarkers for the Severity of COPD. *Int J Chron Obstruct Pulmon Dis* 2022, 17:1537-1552.

22. Li D, Wu Y, Guo S, Qin J, Feng M, An Y, Zhang J, Li Y, Xiong S, Zhou H *et al*: Circulating syndecan-1 as a novel biomarker relates to lung function, systemic inflammation, and exacerbation in COPD. *Int J Chron Obstruct Pulmon Dis* 2019, 14:1933-1941.

23. Macready DM, Johnson LR, Pollard RE: Fluoroscopic and radiographic evaluation of tracheal collapse in dogs: 62 cases (2001-2006). *J Am Vet Med Assoc* 2007, 230(12):1870-1876.

24. Rosenheck S, Davis G, Sammarco CD, Bastian R: Effect of Variations in Stent Placement on Outcome of Endoluminal Stenting for Canine Tracheal Collapse. *J Am Anim Hosp Assoc* 2017, 53(3):150-158.

25. Kwon JW, Moon JY, Kim SH, Song WJ, Kim MH, Kang MG, Lim KH, Lee SH, Lee SM, Lee JY *et al*: Korean version of the Cough Symptom Score: clinical utility and validity for chronic cough. *Korean J Intern Med* 2017,

32(5):910-915.

26. Zhan W, Zhang L, Jiang M, Chen M, Yuan X, Sun J, Xu P, Wu F, Zhang C, Luo W *et al*: A new simple score of chronic cough: cough evaluation test. *BMC Pulmonary Medicine* 2020, 20(1):68.

27. Johnson WK, Mauderly JL, Hahn FF, Muggenburg BA: Lung function and morphology of dogs after sublethal exposure to nitrogen dioxide. *J Toxicol Environ Health* 1982, 10(2):201-221.

28. Swimmer RA, Rozanski EA: Evaluation of the 6-Minute Walk Test in Pet Dogs. *Journal of Veterinary Internal Medicine* 2011, 25(2):405-406.

29. Rubin JA, Holt DE, Reetz JA, Clarke DL: Signalment, clinical presentation, concurrent diseases, and diagnostic findings in 28 dogs with dynamic pharyngeal collapse (2008-2013). *J Vet Intern Med* 2015, 29(3):815-821.

30. Maggiore AD: Tracheal and Airway Collapse in Dogs. *Veterinary Clinics of North America: Small Animal Practice* 2014, 44(1):117-127.

31. Schwartz AR, Eisele DW, Smith PL: PHARYNGEAL AIRWAY OBSTRUCTION IN OBSTRUCTIVE SLEEP APNEA: Pathophysiology and Clinical Implications. *Otolaryngologic Clinics of North America* 1998, 31(6):911-918.

32. Petrof BJ, Pack AI, Kelly AM, Eby J, Hendricks JC: Pharyngeal myopathy of loaded upper airway in dogs with sleep apnea. *J Appl Physiol (1985)* 1994, 76(4):1746-1752.

33. Smith JA, Aliverti A, Quaranta M, McGuinness K, Kelsall A, Earis J, Calverley PM: Chest wall dynamics during voluntary and induced cough in healthy volunteers. *J Physiol* 2012, 590(3):563-574.

34. Hara Y, Teshima K, Seki M, Asano K, Yamaya Y: Pharyngeal contraction secondary to its collapse in dogs with brachycephalic airway syndrome. *The Journal of veterinary medical science* 2019, 82.

35. Ferasin L, Crews L, Biller DS, Lamb KE, Borgarelli M: Risk factors for coughing in dogs with naturally acquired myxomatous mitral valve disease. *J Vet Intern Med* 2013, 27(2):286-292.

36. Singh MK, Johnson LR, Kittleson MD, Pollard RE: Bronchomalacia in dogs with myxomatous mitral valve degeneration. *J Vet Intern Med* 2012, 26(2):312-319.

37. Moritz A, Schneider M, Bauer N: Management of advanced tracheal collapse in dogs using intraluminal self-expanding biliary wallstents. *J Vet Intern Med* 2004, 18(1):31-42.

38. Dabanoğlu I, Ocal MK, Kara ME: A quantitative study on the trachea of the dog. *Anat Histol Embryol* 2001, 30(1):57-59.

39. Bottero E, Bellino C, De Lorenzi D, Ruggiero P, Tarducci A, D'Angelo A, Gianella P: Clinical evaluation and endoscopic classification of bronchomalacia in dogs. *J Vet Intern Med* 2013, 27(4):840-846.

40. Nafe LA, Robertson ID, Hawkins EC: Cervical lung lobe herniation in dogs identified by fluoroscopy. *The Canadian veterinary journal = La revue veterinaire canadienne* 2013, 54 10:955-959.

41. Feist JH, Johnson TH, Wilson RJ: Acquired Tracheomalacia: Etiology and Differential Diagnosis. *Chest* 1975, 68(3):340-345.

42. Demedts IK, Brusselle GG, Bracke KR, Vermaelen KY, Pauwels RA: Matrix metalloproteinases in asthma and COPD. *Curr Opin Pharmacol* 2005, 5(3):257-263.

43. MacNee W: Systemic inflammatory biomarkers and co-morbidities of chronic obstructive pulmonary disease. *Ann Med* 2013, 45(3):291-300.

44. Wei YY, Zhang DW, Ye JJ, Lan QX, Ji S, Sun L, Li F, Fei GH: Interleukin-6 neutralizing antibody attenuates the hypersecretion of airway mucus via inducing the nuclear translocation of Nrf2 in chronic obstructive pulmonary disease. *Biomed Pharmacother* 2022, 152:113244.

45. Rincon M, Irvin CG: Role of IL-6 in Asthma and Other Inflammatory Pulmonary Diseases. *International Journal of Biological Sciences* 2012, 8(9):1281-1290.

46. Bahramabadi R, Yousefi-Daredor H, Rezaeinejad S, Rezayati M, Arababadi MK: Down-regulation of transforming growth factor-beta and interleukin-6 serum levels in the idiopathic chronic obstructive pulmonary disease. *Am J Clin Exp Immunol* 2022, 11(3):45-50.

47. Wright JR: Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 2005, 5(1):58-68.

48. Sone K, Akiyoshi H, Shimizu J, Cao Z, Li Y, Tanaka T, Hayashi A, Sugii S, Ohashi F: Surfactant protein-A concentration in sera from dogs with pulmonary parenchymal diseases. *J Vet Med Sci* 2013, 75(6):685-691.

49. Dy ABC, Tanyaratsrisakul S, Voelker DR, Ledford JG: The Emerging Roles of Surfactant Protein-A in Asthma. *J Clin Cell Immunol* 2018, 9(4).

50. Parimon T, Yao C, Habiel DM, Ge L, Bora SA, Brauer R, Evans CM, Xie T, Alonso-Valenteen F, Medina-Kauwe LK *et al*: Syndecan-1 promotes lung fibrosis by regulating epithelial reprogramming through extracellular vesicles. *JCI Insight* 2019, 5(17).

51. Li Q, Park PW, Wilson CL, Parks WC: Matrilysin Shedding of Syndecan-1 Regulates Chemokine Mobilization and Transepithelial Efflux of Neutrophils in Acute Lung Injury. *Cell* 2002, 111(5):635-646.

52. Brauer R, Ge L, Schlesinger SY, Birkland TP, Huang Y, Parimon T, Lee V,

McKinney BL, McGuire JK, Parks WC *et al*: Syndecan-1 Attenuates Lung Injury during Influenza Infection by Potentiating c-Met Signaling to Suppress Epithelial Apoptosis. *Am J Respir Crit Care Med* 2016, 194(3):333-344.

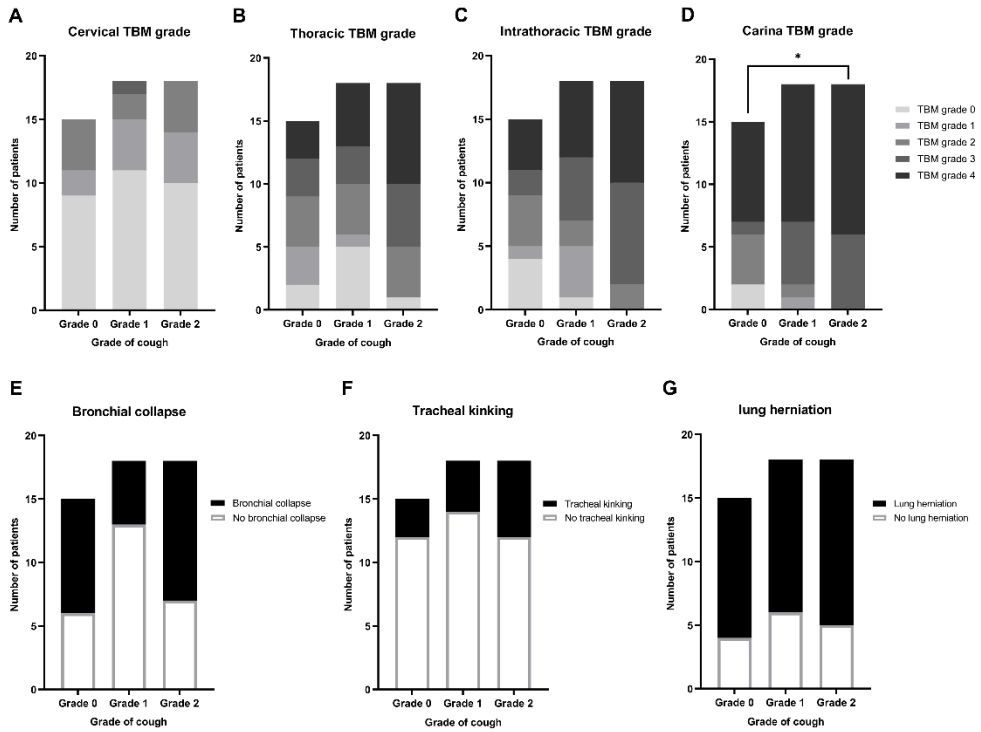

Figure 1. Fluoroscopic characteristics of each cough grade groups. The proportion of dogs with (**A**) cervical TBM grade; (**B**) thoracic TBM grade; (**C**) intrathoracic TBM grade, and (**D**) carinal TBM grade in each cough grade group is demonstrated. Additionally, proportion of presence or absence of (**E**) bronchial collapse; (**F**) tracheal kinking, and (**G**) lung herniation in each cough grade group is demonstrated.

The grade of cough are as follows: grade 0, no cough; grade 1, <3 times a day; and grade 2, ≥3 times a day or ≥5 minutes of cough at a time, or severe cough with cyanosis.

The TBM grade was evaluated by radiologists based on the percentage reduction in the luminal diameter as follows: grade 1, 0–25%; grade 2, 25–50%; grade 3, 50–75%; and grade 4, > 75%.

*The TBM grade of the carinal region increased significantly with the grade of cough ($p < 0.05$)

Abbreviations: TBM, Tracheobronchomalacia

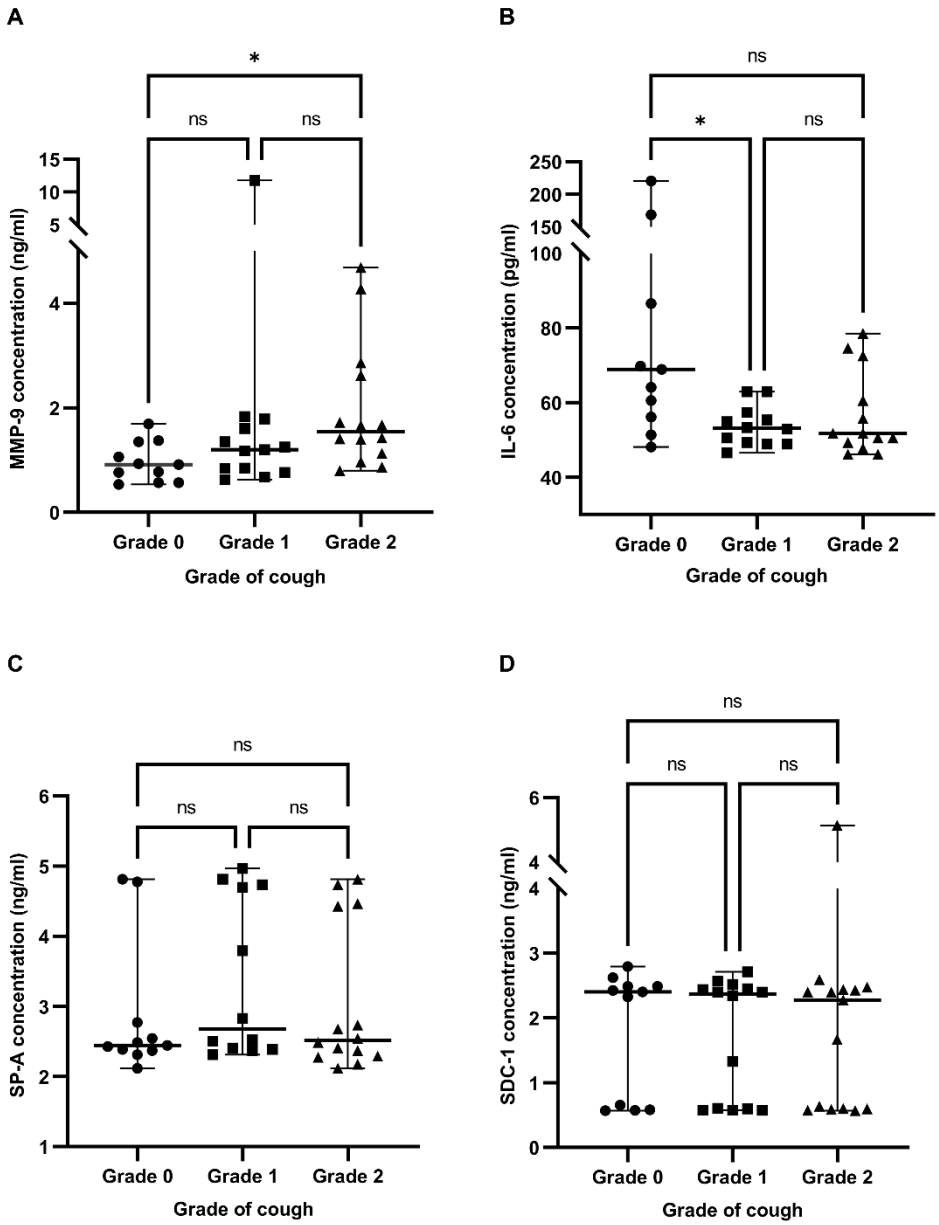

Figure 2. Comparison of the serum levels of MMP-9, IL-6, SP-A, and SDC-1 among the cough grade groups. (**A**) The serum level of MMP-9 was significantly higher in the grade 2 group than that in the grade 0 group ($p < 0.05$). (**B**) The serum level of IL-6 was significantly decreased in the grade 1 group than that in the grade 0 group ($p < 0.05$). (**C**) The serum level of SP-A had no relationship with the grade

of cough. (**D**) The serum levels of SDC- 1 had no relationship with the grade of cough.

* $p < 0.05$ by Kruskal–Wallis test.

Abbreviations: MMP-9, Matrix metalloproteinase-9; IL-6, Interleukin-6; SP-A, Surfactant protein A; SDC-1, Syndecan-1

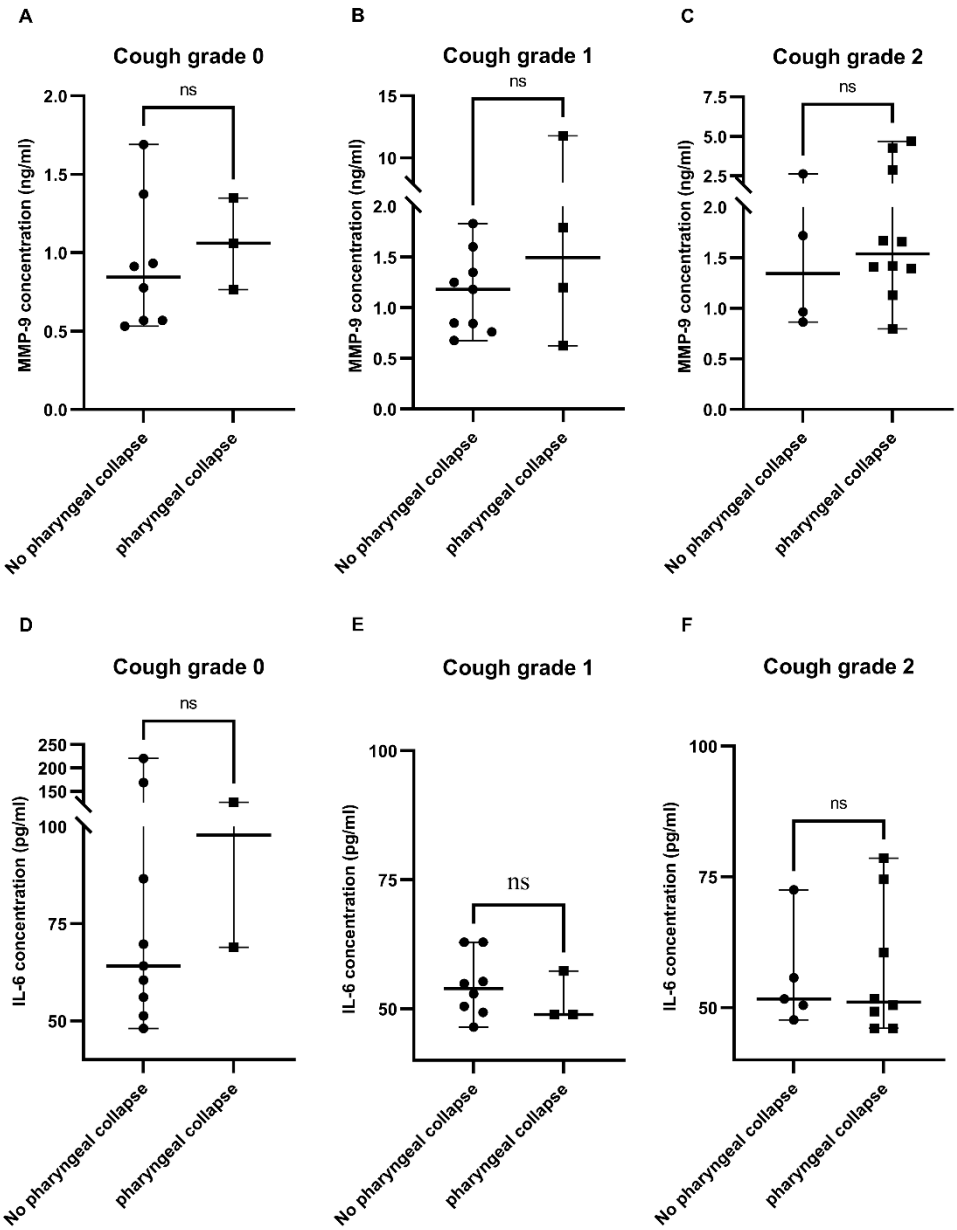

Figure 3. Differences in the serum levels of MMP-9 and IL-6 between dogs with and without concurrent pharyngeal collapse in each cough grade group. Regardless of the presence of pharyngeal collapse, there are no significant differences in the serum levels of MMP-9 in (**A**) the grade 0 group; (**B**) the grade 1 group, and (**C**) the grade 2 group. Similarly, regardless of the presence of pharyngeal

collapse there were no significant differences in the serum levels of IL-6 in (**D**) the grade 0 group; (**E**) the grade 1 group, and (**F**) the grade 2 group.

Abbreviations: MMP-9, Matrix metalloproteinase-9; IL-6, Interleukin-6

Table 1. Signalments, comorbidities, medications, and clinical history of the dogs with TBM in each cough grade group

Characteristic	Grade 0 (n=15)	Grade 1 (n=18)	Grade 2 (n = 18)
Age (years) (median, range)	12 (7–17)	12 (4–16)	11.5 (6–15)
Sex, n (%)			
Male	0 (0%)	1 (5.6%)	0 (0%)
Male castrated	8 (53.3%)	8 (44.4%)	12 (66.7%)
Female	1 (6.7%)	0 (0%)	2 (11.1%)
Female spayed	6 (40%)	9 (50%)	4 (22.2%)
Breed (n)	Toy and Miniature Poodle (5) Pomeranian (3) Maltese (3) Mixed (2) Shihtzu (1) Spitz (1)	Pomeranian (6) Maltese (5) Toy and Miniature Poodle (2) Mixed (2) Shihtzu (1) Silky terrier (1) Beagle (1)	Pomeranian (6) Maltese (5) Chihuahua (4) Toy and Miniature Poodle (1) Shihtzu (1) Yorkshire terrier (1)
Comorbidity, n (%)			
No comorbidity	0 (0%)	1 (5.6%)	1 (5.6%)
MMVD	10 (66.7%)	14 (77.8%)	12 (66.7%)
B1	5 (33.3%)	1 (5.6%)	1 (5.6%)
B2	3 (20%)	6 (33.3%)	4 (22.2%)
C	2 (13.3%)	7 (38.9%)	7 (38.9%)
Medication, n (%)			
None	10 (66.7%)	7 (38.9%)	3 (16.7%)
Theophylline	5 (33.3%)	11 (61.1%)	15 (83.3%)
Codeine	0 (0%)	4 (22.2%)	10 (55.6%)
≥ 3 drugs	0 (0%)	2 (11.1%)	5 (27.8%)

Clinical history, n (%)			
< 3 months	5 (33.3%)	5 (27.8%)	8 (44.4%)
3–6 months	1 (6.7%)	1 (5.6%)	0 (0%)
> 6 months	9 (60%)	12 (66.7%)	10 (55.6%)

Table 2. Serum levels of MMP-9, IL-6, SP-A, and SDC-1 in each cough grade groups

Serum factors	Median (range)			*p*-value
	Grade 0	Grade 1	Grade 2	
MMP-9 (ng/mL)	0.91 (0.53-1.69)	1.20 (0.62-11.78)	1.54 (0.80-4.69)	**0.0144**
IL-6 (pg/mL)	68.93 (48.09-220.42)	53.10 (46.49-62.92)	51.70 (46.09-78.55)	**0.0205**
SPA (ng/mL)	2.45 (2.12-4.81)	2.68 (2.31-4.97)	2.51 (2.12-4.81)	0.46
SDC-1 (ng/mL)	2.40 (0.57-2.79)	2.37 (0.57-2.71)	2.28 (0.57-5.14)	0.88

Abbreviations: MMP-9, Matrix metalloproteinase-9; IL-6, Interleukin-6; SP-A, Surfactant protein A; SDC-1, Syndecan-1

7. 국문초록

기관기관지연화증에 이환된 개에서 기침의 중증도와 투시검사에 기초한 임상적 특징과 MMP-9의 평가

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기관기관지연화증은 기침을 주증으로 하는 개의 흔한 질환이지만, 기관기관지연화증에 이환된 개에서 기침의 중증도를 객관적으로 평가할 수 있는 혈청 바이오마커에 대해서는 거의 알려져 있지 않다. 이 연구의 목적은 기관기관지연화증에 이환된 개에서 기침의 중증도와 임상적 특징, 투시검사 결과 및 새로운 혈청 바이오마커 사이의 관계를 평가하는 것이

다.

투시검사 결과와 임상 증상에 근거하여 기관기관지연화증으로 진단되고 보호자가 있는 51마리의 개가 이 단면 연구에 참여하였다. 기침의 정도(기침의 등급: 0, 1, 2)에 따라 해당 개들을 세 그룹으로 나누었다. 각 그룹간 환자 정보, 병발질환 및 투시검사 결과들을 비교했다. 모든 그룹에서 혈청 matrix metalloproteinase-9 (MMP-9), interleukin-6 (IL-6), surfactant protein-A (SP-A) 및 syndecan-1 (SDC-1)의 농도를 측정하였다.

그룹 간에 연령, 품종, 성별 및 임상 병력의 유의한 차이는 관찰되지 않았다. 기침의 중증도 심화시 인두 허탈이 병발되어 있는 개체가 유의하게 증가했다($p < 0.05$). 투시검사 결과 중, 기관분기부의 기관기관지연화증 등급이 기침의 중증도 심화에 따라 유의하게 지속적으로 증가하였다($p < 0.05$). 혈청 MMP-9 농도는 기침 2등급 그룹에서 기침 0등급 그룹보다 유의하게 높았다($p < 0.05$). 혈청 IL-6 농도는 기침 1등급 그룹에서 기침 0등급 그룹보다 유의하게 낮았다($p < 0.05$). 혈청 SP-A와 SDC-1 농도는 그룹 간에 큰 차이가 없었다.

추가적인 연구들이 이루어진다면, 기침의 중증도를 나타내는 객관적인 혈청 바이오마커로 MMP-9을 활용하고, 기관기관지연화증의 병인론을 이해하는데 도움을 받을 수 있을 것이다.

주요어: 개, 기관기관지연화증, 기관허탈, 투시, MMP-9, IL-6

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